Early age-related cognitive impairment in mice lacking cannabinoid CB1 receptors

A. Bilkei-Gorzo*, I. Racz*, O. Valverde†, M. Otto*, K. Michel*, M. Sarstre‡, and A. Zimmer*§

*Laboratory of Molecular Neurobiology, Department of Psychiatry, and ‡Department of Neurology, University of Bonn, Sigmund-Freud-Strasse 25, 53105 Bonn, Germany; and †Laboratori de Neurofarmacologia, Departament de Ciències Experimentales i de la Salut, Universitat Pompeu Fabra, Carrer del Doctor Aiguader, 80, 08003 Barcelona, Spain

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The molecular mechanisms contributing to the normal age-related decline of cognitive functions or to pathological learning and memory impairment are largely unknown. We demonstrate here that young mice (6–7 weeks) with a genetic deletion of the cannabinoid CB1 receptor performed as well as WT mice, or often better, in a number of learning and memory paradigms, including animal models of skill-learning, partner recognition, and operant conditioning. In contrast, the performance of mature mice (3–5 months) lacking CB1 receptors was much worse than that of age-matched WT animals. In most tests, these mice performed at the same level as old animals (14–17 months), suggesting that the decline in cognitive functions is accelerated in the absence of CB1 receptors. This rapid decline in CB1-deficient animals is accompanied by a loss of neurons in the CA1 and CA3 regions of the hippocampus.

aging | gene knockout | learning | hippocampus | memory

Aging is associated with a decline of cognitive performance in humans (1) and animals (2, 3). However, as in all age-related health issues, there is a wide spectrum of potential outcomes: Although many senior citizens still enjoy their cognitive abilities at an advanced age, others, especially those who suffer from neurodegenerative disorders such as Alzheimer's disease, may show signs of cognitive impairment early in their life. In any case, the increasing average lifespan of the human population does result in a growing number of individuals with cognitive deficits, providing an enormous social and economical challenge to society. This challenge can only be met by developing innovative methods for the treatment and prevention of cognitive impairment, based on a better understanding of the normal physiological and accelerated pathological aging process of the brain.

In the present study, we have analyzed the role of the endocannabinoid system in the age-related decline of learning and memory functions. Cannabinoids are aromatic hydrocarbon compounds from the hemp plant *Cannabis sativa* and include the major psychoactive cannabinoid Δ^9-tetrahydrocannabinol (THC). THC exerts its psychotropic effects by activating the cannabinoid CB1 receptor, which is probably one of the most abundant G protein-coupled receptors in the mammalian brain.

Numerous studies have shown that acute cannabinoid exposure detrimentally affects learning and memory functions (4). In humans, the severity of the cognitive impairment is correlated with the difficulty of the task, with recognition memory being particularly sensitive to disruption by cannabinoids. In animals, cannabinoid administration impairs spatial (5, 6) and working memory (7) and memory consolidation (8). Acute pharmacological blockade of the CB1 receptor has a beneficial effect on the memory. SR141716A, a widely used CB1 receptor antagonist, not only prevented tetrahydrocannabinol-induced memory deficits (9, 10) but, when applied alone, also improved retention of spatial memory (11) and social recognition (12) and reduced memory deficits in aged rodents (12). However, the effects of long-term pharmacological manipulations of the endocannabinoid system are not clear. Most of the recent studies, for example, did not confirm previous evidence for cognitive impairment in chronic cannabis users.

The pharmacological results are supported by behavioral analyses of mice with a deletion of the CB1 receptor gene *Cnr1* (henceforth referred to as *Cnr1*$^{-/-}$ mice), which were mostly performed with young mice. Collectively, these studies show that some cognitive or memory functions may be altered in *Cnr1*$^{-/-}$ mice when compared with WT mice, because they show enhanced memory retention in the object-recognition task (13, 14), a deficit in reversal learning in the water-maze test (15), and a delayed extinction learning in a fear-conditioning paradigm (16, 17).

The cannabinoid system undergoes characteristic age-related changes. Old rats, when compared with young animals, showed reduced CB1 receptor densities and mRNA expression levels in many brain areas, most prominently in the basal ganglia and in the cerebellum (18, 19). The brainstem of aged rats, in contrast, showed a substantial increase in CB1 mRNA levels. In the cortex, the age-dependent change in CB1 receptor binding or mRNA expression seems to be region-specific; a decrease (19), no change (20), and an increase (21) has been described in different areas. The concentration of endocannabinoids also shows a region specific reduction in aged animals, although it is rather modest (14, 20). The comparison of 26- to 48-week-old mice with 6- to 10-week-old mice did not reveal any differences in CB1 endocannabinoid levels but showed a reduced receptor coupling in the limbic forebrain of older animals. The functional consequences of these age-related changes remain to be shown, but it has been suggested that they contribute to behavioral changes observed in aged animals, such as the age-dependent decline in food and alcohol intake.

Here, we compared the age-related decline in learning and memory functions in WT mice and in *Cnr1*$^{-/-}$ animals. Although young CB1-deficient mice performed as well as, or better than, WT animals in most memory tasks, we found a very surprising accelerated decline of cognitive functions in mature animals, accompanied by neuronal cell loss in the hippocampus.

Methods

Animals. Experiments were carried out with young (6–8 weeks old), mature (3–5 months old), and old (14–17 months old) male *Cnr1*$^{-/-}$ and *Cnr1*$^{+/+}$ mice on a congenic C57BL6/J background (22). For some experiments, we also used *Cnr1*$^{-/-}$ mice on a CD1 genetic background (43), which were generously provided by Catherine Ledent (Université Libre de Bruxelles, Brussels). All experiments with C57BL6/J-*Cnr1*$^{-/-}$ mice (and WT controls) were performed at University of Bonn; the experiments with CD1-*Cnr1*$^{-/-}$ mice (and WT controls) were performed at Universitat Pompeu Fabra. Animals received water and food *ad*

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Abbreviation: CI, confidence interval.

§To whom correspondence should be addressed. E-mail: a.zimmer@uni-bonn.de.

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libitum except during the operant conditioning period. Mice were housed in groups of three to five and kept in a reversed light–dark cycle (with a dark period between 9 and 18 h). Experiments were carried out in the active phase of the animals in a dimly lit, low-noise environment. The experimenter was blind to the genotype, but the difference between age groups was evident. Care of the animals and conduct of all experiments followed the guidelines of European Communities Directive 86/609/EEC and the 1998 German Animal Protection Law regulating animal research.

Open-Field Test. Mice were placed into the center of a dimly lit (20–30 lux) chamber of the open-field apparatus (44 × 44 × 30 cm). Movements of the animals were tracked by an automatic monitoring system (TSE Systems, Bad Homburg, Germany) for 20 min. Horizontal motor activity was evaluated by calculating the distance that the animals traveled. Mean value and standard error was calculated for each group. Groups were compared by two-way ANOVA (age × genotype) followed by the Student–Newman–Keuls test.

Skill-Learning on a Rotarod. Animals were placed gently onto a rod of a rotarod device. The speed of the rod accelerated from 4 to 20 rpm (acceleration was 1 rpm/s) and remained constant until the end of the trial. We registered the time until the animals fell down. The cutoff time was 90 s. The animals were allowed to rest for 1 min between two trials. The animals were tested until they showed no further improvement in the performance, which means the variation between three consecutive trials was <30%. The time-dependent change in performance was best fitted by a sigmoid curve derived from the Hill equation. We expressed the speed of learning as the number of trials [with 95% confidence intervals (CIs)] necessary to reach 50% of the maximal performance (i.e., the inflection point of the fitted curve using the Hill equation).

Partner Recognition Test. The test was conducted in an open-field arena (44 × 44 cm) in a dimly lit, sound-isolated environment. The floor of the arena was covered with sawdust saturated with the odor of mice. Initially, the animals were habituated to the arena for 5 min daily for 3 days. The experiments started on the fourth day and consisted of two sessions. First, we put the animals into the familiar arena, where a juvenile (3–4 weeks) male DBA/2J-Penk1$^{-/-}$ mouse was present. We have previously demonstrated that these mice have a very low social activity (23). They will only rarely initiate social contacts; thus, the overall level of social interactions will largely depend on the other partner. The activity of mice was videotaped for 5 min, and the time spent with investigation of the partner was calculated by using THE OBSERVER software (Noldus Information Technology, Wageningen, The Netherlands). In the next session, the test was repeated with the same partners and in the same arena. The time interval between the sessions in the following experiments was 1, 4, 8, 16, and 24 h. For each time interval, a separate experiment was carried out; the animals were left undisturbed between two experiments for 24 h. The partner was always new for the test animal in the consecutive experiments. A significant reduction in the time the animals spent with social interactions in the second presentation (Student's paired *t* test) was considered as an indication that the animals recognized the partner. The test was repeated until the animals failed to recognize the partner in the second presentation.

Operant Conditioning Test. Animals received only 80% of the food quantity they consumed normally during the test, except for one control experiment where WT mice received 80% or 90% of the food quantity. Test cages (17 × 17 × 17 cm) were made from transparent plastic material and placed into a larger (50 × 40 × 70 cm) wooden box. Each cage contained a nose-poke sensor, a feeder, and a lamp for visual cues. The cages were connected to a computer-regulated central unit (TSE Systems). Animals were placed individually into the cages, and the number of nose-pokes into the sensor hole was registered for 20 min. Each nose-poke resulted in a delivery of a 20-mg food pellet [fixed ratio schedule 1 (FR1), Bioserv, Frenchtown, NJ). The timeout period was 5 s and was signaled with a yellow lamp. The animals were tested daily until the variation between the responses on 3 consecutive days was <30%, but for a maximum for 17 days. Means and SDs of nose-pokes were calculated daily for each group. The speed of learning was established as the number of experiments (days) needed to reach 50% of the maximal performance using the Hill equation.

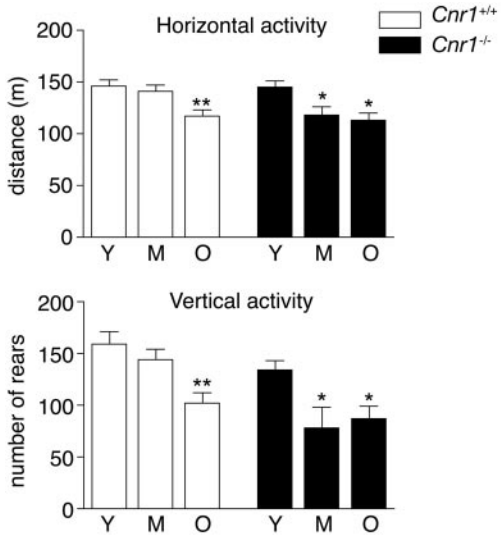

Fig. 1. Open-field test. The activity of young, mature, and old mice was evaluated in the open field in a dimly lit environment. Both the horizontal and the vertical activity showed an age-dependent decrease in both genotypes; this decrease was significant in the old age group in *Cnr1*$^{+/+}$ animals and in mature and old *Cnr1*$^{-/-}$ mice. In young animals, we found a small reduction of vertical, but not horizontal, activity in *Cnr1*$^{-/-}$ mice. Each column represents the mean (+SEM), $n = 10$. *, $P < 0.05$; **, $P < 0.01$ compared with the young age group (Student–Newman–Keuls test). Y, young; M, mature; O, old.

Immunohistochemistry and Neuronal Cell Counts. Brains were rapidly isolated after decapitation of the animals and frozen in isopentane cooled with dry ice, and 8-μm sections were cut by using a cryostat. The frozen sections from mature WT and transgenic mice were fixed with methanol, blocked with goat serum, and incubated overnight at 4°C with monoclonal antibody against Neu-N (1:500, Chemicon). Immunohistochemistry was performed by using the avidin-biotin peroxidase complex method (ABC-Kit, Vector Laboratories) with 3,3′-diaminobenzidine tetrahydrochloride as chromogen. For quantitative analysis of hippocampal sections, serial coronal sections of one hemisphere were examined. All images were acquired by using a standard light and immunofluorescence microscope (Nikon Eclipse E-800) connected to a digital camera (DXC-9100P, Sony, Tokyo) and a PC system with LUCIA 32G 4.11 imaging software (Laboratory Imaging, Düsseldorf, Germany). Neuronal density was determined in the CA1, CA2/CA3, and CA3 regions of the hippocampus. Neurons (principal neurons and interneurons) were counted in 100 × 50-μm quadrants. At least three independent sections from each mouse were evaluated. The neuronal densities were calculated from the average values as number of neurons per mm^2. Groups were compared by using an unpaired *t* test ($n = 8$ or 4 for old *Cnr1*$^{+/+}$ animals).

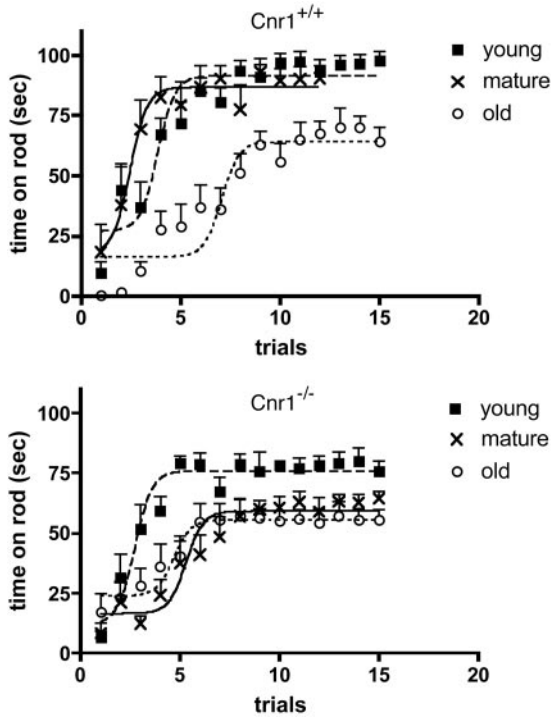

Fig. 2. Skill-learning on a rotarod. Time spent on the rotating rod is presented as a function of trials. Symbols represent the mean value (+SEM) of 8–10 animals. The inflexion point of a sigmoid curve derived from the Hill equation indicates the performance of the task acquisition. Young and mature *Cnr1*$^{+/+}$ animals show a similar performance. Old *Cnr1*$^{+/+}$ mice learn the task less well, and they show a lower maximal performance. Young *Cnr1*$^{-/-}$ mice also readily learn the task, whereas mature and old *Cnr1*$^{-/-}$ mice show a similarly poor performance in this test.

Results

Open-Field Test. We have previously shown that *Cnr1*$^{-/-}$ mice were less active than *Cnr1*$^{+/+}$ animals in the open-field test under regular laboratory light conditions. This phenotype seems to be related to the adversity of the experimental situation, because we now found a much smaller difference between the genotypes under low light conditions (i.e., in a less stressful environment). *Cnr1*$^{-/-}$ mice still showed a reduction in vertical ($F_{1,48} = 11.60$; $P < 0.001$) but not in horizontal ($F_{1,51} = 3.00$; $P > 0.05$) motor activity compared with *Cnr1*$^{+/+}$ controls (Fig. 1). We found in both genotypes an age-related decrease of horizontal ($F_{2,51} = 10.14$; $P < 0.001$) and vertical ($F_{2,48} = 8.67$; $P < 0.001$) activity. There was no significant interaction between genotype and age for either of these two parameters (horizontal activity: $F_{2,51} = 1.61$, $P > 0.05$; vertical activity: $F_{2,48} = 2.16$, $P > 0.05$). However, comparing separately within the genotypes the activity of different age groups by using one-way ANOVA (followed by a Student–Newman–Keuls test), we found that mature *Cnr1*$^{+/+}$ mice behaved similarly to young mice, whereas mature *Cnr1*$^{-/-}$ mice behaved like old animals.

Skill-Learning on the Rotarod. We next tested the animals in a skill-learning paradigm on the rotarod. As shown in Fig. 2, both young and mature *Cnr1*$^{+/+}$ animals readily learned this task, and many were able to balance on the rotating beam for almost the entire time of each of the 90-s sessions after a few trials. Although both age groups performed similarly well after they had learned the task, we found a small but significant difference in the number of trials until they reached a half-maximal performance (speed of learning), indicated by nonoverlapping CIs (young: 3.8 trials, CI = 3.7–4.0; mature: 2.5 trials, CI = 2.4–2.6). This result shows that mature animals had a slightly higher speed of learning.

In contrast, old *Cnr1*$^{+/+}$ mice had great difficulty with the rotarod task. They had a much lower speed of learning than the younger animals (7.1 trials, CI = 6.9–7.3), and even experienced mice rarely reached the cutoff time.

In mice without CB1 receptors, only young animals performed the task well enough to reach the cutoff time. Indeed, young *Cnr1*$^{-/-}$ mice required significantly fewer trials to reach a half-maximal performance than WT *Cnr1*$^{+/+}$ animals. This result was confirmed with CB1-deficient mice on a CD1 genetic

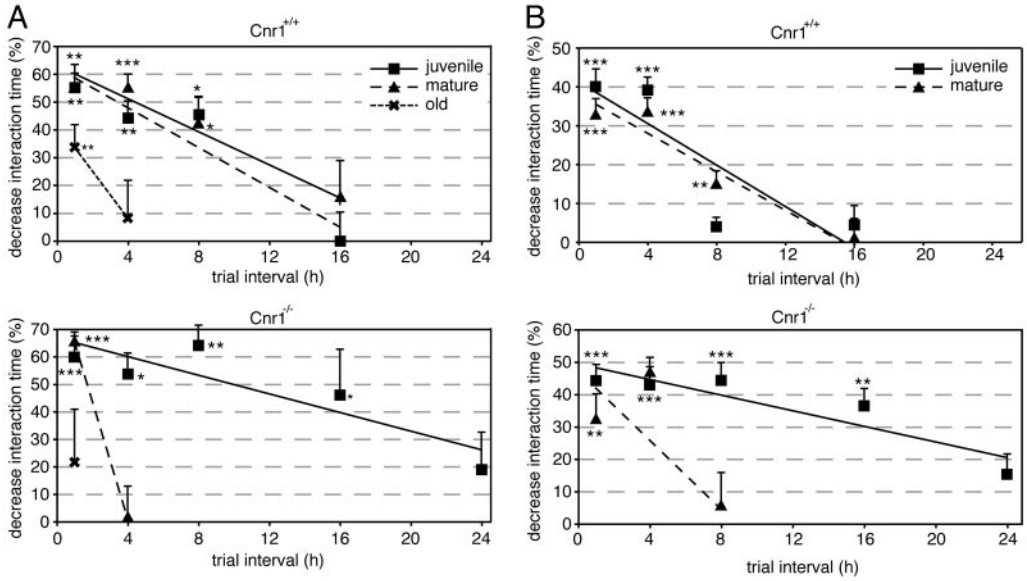

Fig. 3. Partner recognition test with animals from C57BL/6J (*A*) and CD1 (*B*) genetic backgrounds. Reduction in the duration of exploratory social contacts between the first and second trials is indicated as a function of intertrial time. Symbols represents the mean value (+SEM) of 8–10 animals. Sign of recognition is a significant difference in social time between the first and second presentation (Student's paired *t* test). The duration of social memory was ≈8 h in young and mature *Cnr1*$^{+/+}$ mice but only 1 h in old *Cnr1*$^{+/+}$ animals. Young *Cnr1*$^{-/-}$ animals were even able to recognize their partner 16 h after the first presentation. The maximum duration of recognition is 1 h in mature mice and <1 h in old *Cnr1*$^{-/-}$ mice. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, first vs. second presentation (Student's paired *t* test).

background (C57BL/6-*Cnr1*$^{-/-}$: 2.7 trials, CI = 2.6–2.8; C57BL/6-*Cnr1*$^{+/+}$: 3.8 trials, CI = 3.7–4.0; CD1-*Cnr1*$^{-/-}$: 2.6 trials, CI = 2.1–3.0; CD1-*Cnr1*$^{+/+}$: 3.8 trials, CI = 3.1–4.4). However, we found a striking performance deficit in mature *Cnr1*$^{-/-}$ mice, which were almost indistinguishable from old knockout animals in this task. Mature and old *Cnr1*$^{-/-}$ mice also showed a reduced speed of learning (young: 2.7 trials, CI = 2.6–2.8; mature: 5.3 trials, CI = 5.1–5.4; old: 4.7 trials, CI = 4.6–5.8). Thus, these results point to a specific age-related performance deficit in *Cnr1*$^{-/-}$ animals.

Partner Recognition Test. This paradigm evaluates the ability of animals to recognize a previously seen partner. *Cnr1*$^{+/+}$ and *Cnr1*$^{-/-}$ animals are exposed to a juvenile con-species male on two consecutive sessions with variable time intervals. If these animals recognize the partner, they spend less time with social investigations in the second presentation.

Young and mature WT mice performed similarly well in this test: They readily recognized the partner 1, 4, and 8 h after the first presentation but not after 16 h (Fig. 3*A*). Old WT mice, in contrast, already failed to recognize the partner at 4 h, indicating a clear memory deficit (Fig. 3*A*).

Young *Cnr1*$^{-/-}$ mice showed an even better performance in this test than age-matched WT animals. They still recognized the partner after 16 h, but not after 24 h. However, mature *Cnr1*$^{-/-}$ showed a striking memory deficit: They recognized their partner when tested 1 h after the first exposure to the partner, but not after 4 h. The performance of old *Cnr1*$^{-/-}$ mice was even worse: They failed to recognize the partner after a 1-h trial interval.

Because the genetic background is often discussed as a confounding factor in the expression of knockout phenotypes, we have also repeated this test using young and mature *Cnr1*$^{-/-}$ mice on a CD1 genetic background. As shown in Fig. 3*B*, young and mature *Cnr1*$^{+/+}$ animals performed again almost identically in this test. However, young *Cnr1*$^{-/-}$ mice performed much better than mature animals, and they performed better than young *Cnr1*$^{+/+}$ animals. Mature *Cnr1*$^{-/-}$ mice recognized their partner when tested 4 h after the first exposure, but not after 8 h. In contrast, mature *Cnr1*$^{+/+}$ animals still recognized their partner after 8 h. Thus, the memory deficit observed on the C57BL/6J background is similar on the CD1 background.

Operant Conditioning. The animals from each strain and age group displayed a uniform learning pattern in this test: little improvement during the first few trials, followed by a rapid increase in performance and, finally, a stable performance plateau (Fig. 4). Sigmoid curve fitting using the Hill equation was used to describe the change in performance in the successive experiments. The inflection point served as an indicator of the speed of learning, because it is independent of the maximal performance and thus of the hedonic value of the reward. When we compared the performance of WT mice that received 80% or 90% of their normal food quantity, we found no difference in the number of trials to reach 50% performance (80%: 3.8, CI = 3.4–4.2; 90%: 3.3, CI = 3.2–3.5), although the 80% group showed a higher maximal performance.

The learning ability of young and mature WT animals was similar, although mature mice reached a higher maximal performance. Young animals required 3.8 trials (CI = 3.4–4.2), and mature mice required 3.3 trials (CI = 2.9–3.7), to reach the 50% performance. In contrast, old animals needed almost twice as many trials, with an average of 6.0 (CI = 4.4–7.6), to perform at the same level.

In CB1-deficient mice, we found a significant difference in the operant learning ability between the young and mature age groups: Mature animals needed 6.7 trials (CI = 6.3–7.1), whereas young mice required 4.6 trials (CI = 4.3–4.8), to reach the same level of performance. The difference between mature and old animals in the *Cnr1*$^{-/-}$ strain was less pronounced: old animals required 10.2 trials (CI = 9.9–10.6) to reach the 50% performance level (Fig. 4).

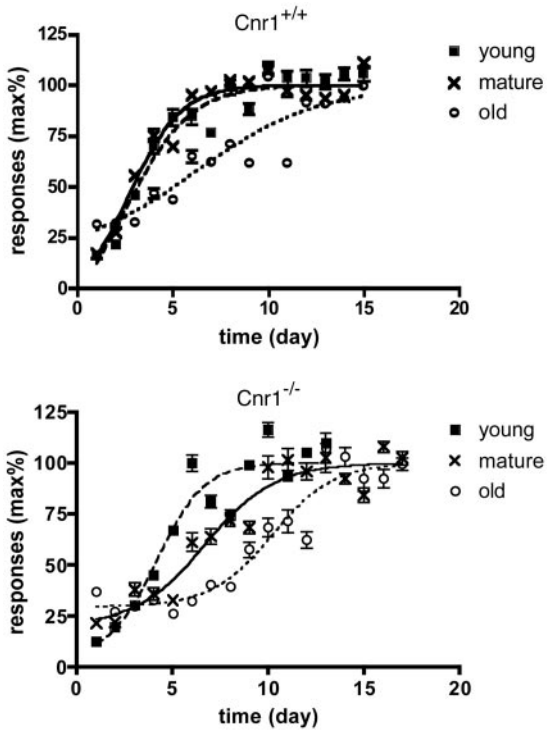

Fig. 4. Operant conditioning. Performance is expressed as percent maximum performance as a function of time. Symbols represent the mean value (±SEM) of 8–13 animals. The sigmoid curve derived from the Hill equation indicates the learning performance during the repetitions. Young and mature *Cnr1*$^{+/+}$ mice showed an almost identical performance, whereas old *Cnr1*$^{+/+}$ animals learned the task more slowly. *Cnr1*$^{-/-}$ mice also readily learned the task, whereas mature and old *Cnr1*$^{-/-}$ animals showed a similarly low performance in this model.

Neuronal Density. To determine whether the accelerated decline of learning and memory functions in CB1-deficient mice was accompanied by pathological changes in brain morphology, we performed a histological analysis of serial brain sections from *Cnr1*$^{-/-}$ and *Cnr1*$^{+/+}$ animals of all age groups. Brains of *Cnr1*$^{-/-}$ mice did not show any apparent gross morphological abnormalities, although we noticed a reduced cell density in the hippocampus. We therefore performed neuronal cell counts after staining with the neuronal cell marker Neu-N in the hippocampal CA1, CA2/3, and CA3 regions and in the dentate gyrus. Indeed, we found a significant reduction in neuronal densities in the CA3 region already in young *Cnr1*$^{-/-}$ mice, as well as in the CA1 region in mature and old *Cnr1*$^{-/-}$ animals (Fig. 5). There were no differences in the CA2/CA3 region or in the dentate gyrus. The genotype difference in the CA3 region appeared to be similar in all age groups, whereas the difference in the CA1 region seemed to increase with age. In old animals, the cell density in the CA1 region of *Cnr1*$^{-/-}$ mice was decreased by almost 70% when compared with *Cnr1*$^{+/+}$ mice (*Cnr1*$^{+/+}$, 2,650 ± 123; *Cnr1*$^{-/-}$, 838 ± 154; $P < 0.001$).

Discussion

As part of the physiological aging process, learning ability also declines with age. This study examines the role of the endocannabinoid system in age-related learning performance and provides unequivocal evidence that the decline is accelerated in the

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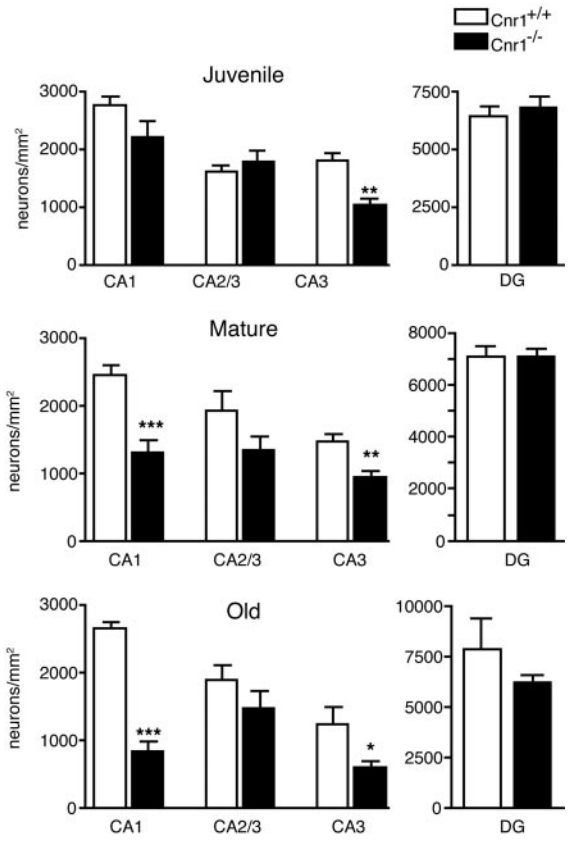

Fig. 5. Neuronal cell densities in the hippocampus. The density of neurons in the CA3 regions was reduced in *Cnr1*$^{-/-}$ mice of all age groups. Significantly lower neuronal densities were also observed in the CA1 region of mature and old *Cnr1*$^{-/-}$ mice. In this region, the neuronal loss seemed to progress with age. Bars represent mean neuronal density expressed as the number of neurons per mm^2 (+SEM), $n = 4–8$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, *Cnr1*$^{-/-}$ vs. *Cnr1*$^{+/+}$ mice (Student's unpaired *t* test).

absence of CB1 receptors. Thus, young *Cnr1*$^{-/-}$ mice performed better than age-matched *Cnr1*$^{+/+}$ mice in the rotarod and partner recognition tests, whereas mature *Cnr1*$^{-/-}$ animals showed significant learning deficits and performed similarly to old mice in most behavioral learning paradigms.

The age-related learning deficit appeared to be generalized rather than related to a specific paradigm. Psychomotor skill-learning, as assessed in a modified version of the rotarod test, involves cortical regions together with the neostriatum and cerebellum (24). Indeed, learning improvement is accompanied by plastic changes in the striatum and motor cortex. "Fast" learning (improvement within the initial training session) involves a recruitment of task-related neurons in both brain structures, whereas the firing pattern is refined during the "slow" learning period (improvement between sessions) (25). Interestingly, it has recently been demonstrated that young C57BL/6J mice performed worse than other mouse strains (NMRI and C57BL/6J × 129OlaHsd) in skill-learning tasks, although they were the best performers at the adult stage (26). In good agreement with this result, we found that mature WT C57BL/6J animals performed this task better than young mice. It was therefore particularly striking to see that mature *Cnr1*$^{-/-}$ mice had a substantially reduced psychomotor performance and performed at a similar level as old mice. It is also noteworthy that old *Cnr1*$^{-/-}$ mice showed a better rotarod performance when compared with old *Cnr1*$^{+/+}$ animals. Because motor coordination skills are an important contributing factor to the performance in this test, it seems possible that sensorimotor performances and cognitive functions are differentially affected by the *Cnr1* mutation.

The operant conditioning paradigm contains elements of working, procedural, and spatial learning. It is well established, and supported by our findings, that old animals have difficulties in the acquisition of operant behaviors (27–29). Indeed, the operant learning ability of *Cnr1*$^{+/+}$ young and mature animals was similar, whereas old mice showed a significant impairment. In *Cnr1*$^{-/-}$ animals, however, we found a continuous age-dependent decrease in performance. *Cnr1*$^{-/-}$ mice are known to eat less than *Cnr1*$^{+/+}$ mice after food deprivation (30, 31) and thus may be less motivated to work for food in the operant behavior paradigm. However, Valverde and colleagues (32) have recently shown that operant behavior for natural rewarding stimuli, such as water and food, was not altered between *Cnr1*$^{+/+}$ and *Cnr1*$^{-/-}$ mice in any of the reinforcement schedules used (FR1 and FR3). In addition, no genotype differences were observed in a progressive ratio schedule of reinforcement between the breaking points obtained in both genotypes for water and food, thus arguing against an altered motivation for these natural stimuli in *Cnr1*$^{-/-}$ animals. Recently, Holter *et al.* (33) have also studied operant behaviors in *Cnr1*$^{-/-}$ mice that were between 11 and 14 weeks of age and thus between the young (6–8 weeks) and mature (14–20 weeks) mice from this study. They demonstrated a slight performance deficit in *Cnr1*$^{-/-}$ mice during the acquisition phase but an equal performance of *Cnr1*$^{+/+}$ and *Cnr1*$^{-/-}$ animals during the end of the training period and the retention phase of the test.

In the social recognition test, old *Cnr1*$^{+/+}$ mice showed a marked deficit in the recognition of the previously seen partner compared with young and mature animals. A similar age-dependent deterioration of social memory was reported in rats (34, 35). We observed again a significant impairment in the social memory task in mature *Cnr1*$^{-/-}$ mice. These animals displayed a normal short-term memory but a complete lack of long-term social memory. This result is contradictory to the previously observed improvement by SR141716A treatment of long-term, but not short-term, social memory performance and the reduction of memory deficits in aged animals with this compound. One possible reason for this discrepancy could be related to the non-CB1-mediated effects of SR141716A, which have been demonstrated repeatedly by using CB1-deficient mouse strains (36, 37). However, we find it more likely that the discrepancy is due to the different physiological effects of the short-term pharmacological blockage vs. long-term genetic ablation of CB1 receptors.

A number of studies have shown that CB1 receptors are expressed in the developing nervous system, and there is some evidence from human and animal studies to suggest that prenatal exposure to cannabinoids affects neurobehavioral development. Thus, it is conceivable that mice develop subtle brain defects in the absence of CB1 receptors and, in consequence, show a poor learning performance. However, young CB1-deficient mice are not impaired in learning and memory tests. In fact, this study, as well as others, strongly suggests that the learning performance is rather improved in young animals in the absence of CB1 receptors. These findings therefore argue against a developmental cause for the learning impairment of mature *Cnr1*$^{-/-}$ mice.

We have therefore considered the possibility that the accelerated decline in learning performance in mature *Cnr1*$^{-/-}$ animals is related to the documented neuroprotective effects of endocannabinoids (38, 39), which are mediated by CB1 receptors (40). Indeed, when we examined the neuronal density in the hippocampus, we found a significant reduction in mature mice in the CA1 or CA3 regions but not in the CA2/CA3 region or in the dentate gyrus, which are known to be differently sensitive to neurotoxic effects (41, 42). These results strongly suggest that the

endocannabinoid system has a neuroprotective function in WT mice that depends on CB1 receptors.

Age-related alterations in the endocannabinoid system have also been studied in young (6–10 weeks) and old (26–48 weeks) mice (20). There were changes neither in tissue levels of endocannabinoids nor in the density of CB1 receptors. However, cannabinoid-stimulated [^{35}S]GTP[γS] binding in the limbic forebrain was higher in young mice compared with old mice, probably due to a reduced coupling of CB1 receptors to G proteins in old animals. In rats, a region-specific decrease in CB1 receptor binding (21) and expression (18) was also reported. It has been suggested that this reduced CB1 receptor function accounts for some age-related changes in cannabinoid-modulated behaviors, such as food and alcohol intake. As a corollary, an age-related impairment in CB1 receptor function may contribute to the normal decline in learning and memory performance in old animals.

Our results also have important medical implications. To the best of our knowledge, long-term effects of CB1 receptor antagonist treatments on learning and memory, age-related decline of cognitive functions, and neurodegenerative processes have not yet been studied. These experiments should be performed, in view of the anticipated long-term use of rimonabant (SR141716A) by patients with eating and addiction disorders.

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